AD-A036 049 ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/1
USE OF COMPUTERS IN THE ANALYSIS OF PLASMA HORMONES. RESULTS WI--ETC(U)
JUN 72 A J VALLERON, G E ROSSELIN

UNCLASSIFIED USAMRIID-MUL-0418 NL

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AD

12 B.S.

TRANSLATION NO.: MUL 0418

TITLE: Use of computers in the analysis of plasma hormones: Results with insulin

AUTHOR(S): Valleron, A.-J. and G.-E. Rosselin

REFERENCE: Ann. Biol. Clin. 29:145-152, 1971

DISTRIBUTION STATEMENT

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U. S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

Fort Detrick, Frederick, Maryland 21701

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Use of computers in the analysis of plasma hormones: Results with insulin		5. TYPE OF REPORT & PERIOD COVERED Translation
		6. PERFORMING ORG. REPORT NUMBER MUL 0418 ✓
7. AUTHOR(s) Valleron, A.-J. and G.-E. Rosselin		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Ann. Biol. Clin 29:145-152, 1971		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS USAMRIID Library, Ft. Detrick, Frederick, Md.		12. REPORT DATE 22 Feb 77
		13. NUMBER OF PAGES 13
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report)
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release: distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Computers
Plasma hormones
Insulin

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

(6)

USE OF COMPUTERS IN THE ANALYSIS OF PLASMA HORMONES. RESULTS WITH INSULIN.

(Apport de l'informatique a l'analyse des hormones dan le plasma. Resultats concernant l'insuline.) (9)

(10) A. J. VALLERON [redacted] G. E. ROSSELIN

Ann. Biol. Clin. 29:145-152, 1971

(Translated by Phebe W. Summers)

(11) 26 Jun[redacted]72

The radioimmunologic method of analysis of protein hormones in complex media (1) presents an interesting advance in diabetology and endocrinology. Several central laboratories have resolved the problems with regard to levels of protein hormones in plasma during analysis annually of several thousand samples. We present here results obtained by information analysis of plasma hormones work, carried out during a study of epidemiologic order in a large group of healthy subjects (2-4). The informational study was applied to determinations of insulin using separation on adsorbant powder. It can be applied equally well to automation of determinations of other hormones such as somatotropic hormone (5) and luteinizing hormone. We will not discuss here the problems posed by automation of the manipulations, we will discuss automation of the necessary calculations for control of quality and management of the results.

Material and Methods

I. Technique of Sampling

The blood samples are drawn and centrifuged using conditions previously described (6).

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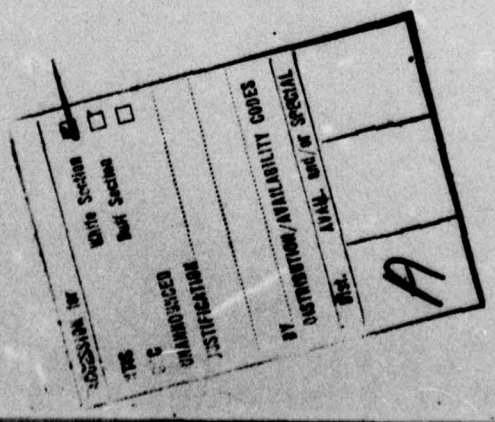

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II. Method of Determination Used

The radioimmunologic assay of hormone is carried out using separation on silicate following the general principles previously presented (7). The technique described for insulin (6), somatotropic hormone (8, 9) and luteinizing hormone (10, 11) have been presented by others. Only the general principles in the informational problem applicable to this method will be mentioned.

Free hormone is absorbed by silicate and after centrifugation, its radioactivity is measured in an automatic counter. Control tubes are measured at the time for a reference or standard curve and for the plasma. They are given as the quantity of iodized hormone adsorbed by the silicate in the absence of immune serum. In the usual experimental conditions, the controls (T) represent 90% of the total radioactivity. It has been verified that this percentage decreases under some conditions thus distorting the results: alteration of the hormone used as tracer at the time of iodination or denaturation of the iodinated hormone at the time of incubation. When the iodinated hormone is bound to an excess of antibody, the percentage of radioactivity is of the order of 10%.

The tubes of the normal or standard curve are obtained by cross-quantities of unlabeled hormone or cold in the presence of the same system of iodinated hormone-antihormone antibody. Under these conditions, the quantity of iodinated hormone adsorbed on the silicate (F) increases and the activity present in the supernatant in the form of a antigen-antibody complex decreases. The ratio of F/T of the quantity of iodinated hormone to the activity of the controls increases as a function of the quantity of

cold hormone. Based on the technique used for separation, it is necessary that the mean of the standard curve should be identical to the mean of plasmas determined. To attain this condition, the curve can be incubated in plasma without hormone, or even a quantity of plasma known to contain small quantities of hormones is added just before the separation replacing an equivalent amount of the incubation mixture. These two techniques, with the immune serum that we used, give similar results. Also, it is easiest to obtain plasma containing a little insulin than plasma not containing all the insulin, the procedure regularly used is that which consists of adding plasma low in hormone before the separation.

III. Calculation Techniques

The programs for treatment of the data have been written in Fortran IV with the exception of the program for decoding the perforated tape coming from the scintillation counter. Classical statistical methods (12) have been used, adjustment of the polynomeals of least squares on observations equally spaced which is utilized for several repetitions in the program was described in particular by Bennet and Franklin (13).

Results

The various informational steps necessary for automation of the determinations are shown schematically in Figure 1. If one follows it the plasma sample from the moment of its being taken to the moment where it results in determination, one can distinguish the following steps: identification of the sample tube of the series, placing in the memory the number of counts, calculation of the quantity of hormone contained in each tube and storage of the results for later use.

I. Identification Step

At the Health Center where the sample is taken, one writes for each plasma sample, on the left side of the card shown in Figure 2, the dates of sampling, name, first name and number of the subject file, the nature of the assay for which the sample was taken, the eventual result and finally the number which is fixed on the tube. This number is used to mark the tubes; at the time when they are introduced into the counter, the right part of the card is filled with the corresponding number at the place in the belt. This procedure eliminates confusing tubes; it allows the laboratory liberty to organize the order of determinations. The second identification problem is the identification of the series of determinations in which the preceding sample is included: date of test, date of incubation, date and specific activity of the iodinated hormone used, date and specificity of the immune serum, date and number of the albumin lot, date and lot of the hormone which was used to establish the standard curve, are noted on a separate card. The group of cards is then transformed to perforated cards for handling by the computer.

II. Calculation of Results Step

To make the calculations possible, a strict plan must be followed, the data coming from the scintillation counter have to be interpreted and, finally, a general program must be written.

A. Protocol of Determination

Preparation of an automation program necessitates definition of a protocol (Table I). A series of determination has been defined as 200 tubes

or more; this quantity is adjustable to the equipment available, but it has been preferable to have a standard curve of this order of numbers of tubes. When more than 200 tubes must be determined, the same protocol is repeated. To establish the standard curve, 8 determinations are used for each in duplicate to allow assessment of the precision of the curve. The chosen determinations are in geometric doubling progression. For insulin, they go from 3μU/ml to 400 μU/ml of plasma, or 0.3 to 40 μU/ml of incubat? The control tubes are equal spaced every 10th on the belt and the test plasma tubes are placed between them..

B. Interpretation of the Data Coming from the Counter

At the end of the counting, numbers of counts corresponding to each tube are punched on a paper tape and become a part of the program, other information is punched at the same time. The perforated tape is decoded and transcribed by a special program onto a magnetic tape for later treatment. The final program uses this magnetic tape for selecting the useful information (number of counts on the channel concerned, etc.); at this transcription step from the perforated tape to the magnetic tape, a list comes to the lab which can briefly verify each item for errors and damage to the perforated tape.

C. Calculation Step

1) Evaluation of the standard curve.

Table II gives the results obtained in an exaple. From the difference observed d_i between the 2 percentages corresponding to the same dose, one can estimate (12) the variance of the percentage of activity or silicate by the quantity

$s^2 = \frac{1}{16} \sum_{i=1}^{8} d_i^2$. In the example treated here one finds $s^2 = 0.70 \times 10^{-4}$ with 8 degrees of freedom.

This supposes that the percentage activity on silicate has a constant variance whatever the dose, and is a reasonable approximation.

The problem of linearization of the standard curve of a radioimmunoassay is often proposed (14). The usual transformations proposed (probit, logit...) do not suffice to linearize the standard curve in our experience (5). Finally, the automatization makes it possible to have confidence in the quality of the method of linearization chosen so as to correct the program for each occurrence. We do not linearize the standard curve but approach it either by polygonal adjustment or by the polygon of least squares of the 4th degree; the log doses being equally space, the use of orthogonal polygons allows easy calculation (13), the polynomial of which can be verified in Figure 3 and fits the facts perfectly. This is the expression of the standard curve which is used by the rest of the program for later calculations. In Figure 3 are shown by dashed lines, the uper and lower limits that can be assigned to the standard curve Y, sup. and Y_1 inf. are calculated by the relationship Y_i sup $= \hat{Y}_i + t_8 s$ and Y_i inf $= \hat{Y}_i - t_8 s$ in which Y_i is the value estimate of the standard curve, and t_i is the Student t for 8 degrees of freedom corresponding to the chosen probability (for a confidence interval having 95 changes in 100 of containing the true value, one has $t_8 = 2.306$). These limits of the standard curve permit giving for each dosage a CL of 95%.

2) Taking account of the variation of the control tubes

The decrease of the radioactivity of iodine in the course of determination, the fluctuations of the measure of the radioactivity and diverse events make the number of total counts of the control tubes vary from one end to the other of the run. One must take into account the systematic variations of these controls, to eliminate the variations of these controls, to eliminate the variations due to imprecision in the number of counts and to develop a procedure capable of measuring the very large variations of these controls which can be interpreted as the smallest number of incidents able to affect the quality of the determinations. Figure 4 shows the variations of the controls during the course of one run. The line trace is the polynomial of least squares. Each sample of plasma will be reported as a theoretical control which is found at the same place and whose value will be calculated by the preceding polynomial. Finally, when the 2 experimental controls on either side of a tube differ one from the other by 75% of the calculated value of the control, a signal appears in the results, asking for verification of the origin of this control variation.

Neighboring controls are very close to each other in value; the principle of calculation of a theoretical control for each standard tube is maintained; still, it is a straight line of least squares which is used for each calculation.

III. Expression of the Results

Results are preserved as a printed document and on magnetic tape for later statistical treatment. The printed results are divided into 3 parts:

one concerns the calculations related to the standard curve and its precision, and the estimations of the standard and plasma controls for each tube; the second concerns the detailed results (fig. 5) where are shown all the identification in the middle of the previous determination, the estimated quantity of insulin and its 95% confidence limits relative to the imprecision on the standard curve, a recall of the number of counts observed in its tube, the neighboring experimental controls and the calculated controls. The third part is a summary of the results (fig. 6) and is printed in several copies. Where the controls are too variable or where an incident has been signaled at the time of manipulation, the results are marked with "à verifier." (to verify)

Comparison between the manual estimation of 96 determinations and the estimation using our program was made: there was very high correlation ($r = 0.99$, $p < 0.001$).

More interesting is the highly significant difference ($p < 0.001$) between the 2 estimates. This difference can be interpreted by the fact that manual estimation does not allow close approximation of the standard curve and the variation in the controls.

Discussion and Conclusions

The method of expressing the standard curve which we propose avoids all the limitations imposed by linearization methods (15).

Another factor for our procedure permits controls throughout the determinations: identification controls of the tubes, control of quality of the information recorded from the counter, control of the stability of

the measure of radioactivity in the counter; the confidence interval calculated for each determination permits comparison of several series of determinations against the same standard curve. The use made of this confidence interval permits ignoring for the first ime on the one hand the error of the hypothesis of equality of the variance as a function of dose (14), on the other, the fact that it is only a reflection of the imprecision of the standard curve. It will still be necessary to bring into the calculation of the confidence interval the imprecision on the number of counts obtained at each determination; this last operation loses nevertheless its importance from the moment where one compares it to the previous calculation.

Finally, it should be noted that the informational application to radioimmunologic determination (15) has the same advantages as those implicated in other methods of analysis. These advantages are of particular interest in radioimmunologic determinations because determinations can be carried out in a large series when plasma specimens have been stored for several months at -20°C. We note in particular the increase in quality of the measurements, the gain in time for the laboratory in taking the counts and the grouping of the calculations.

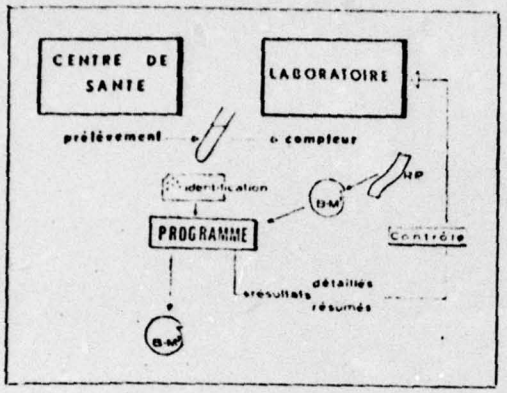

Fig. 1. — Etapes informatiques de l'automatisation des dosages. R.P. : ruban perforé. (perforated tape)

B.M.[1] : bande magnétique sur laquelle sont enregistrées les données sortant du compteur.

B.M.[2] : bande magnétique contenant les dossiers des sujets correspondant aux prélèvements donnés.

AUTOMATISATION DU DOSAGE : Feuille 3

IDENTIFICATION DES PLASMAS

Partie à remplir au moment des prélèvements						Partie à remplir au moment du dosage		
Date du prélèvement						date (rappel)		
Nom	Prénom	N° enquête ou dossier	N° Labo U 55	Temps prélèv.	Remarques	N° tirage au sort ou 2	N° tirage 1 à 100	Date tube plasma 9 contrôle ?
25	36 37	42 43	44 45 46 47 48 49	50	51	67	68 69 70	80

Fig. 2. — Fiche d'identification des tubes.
La partie gauche est remplie au lieu du prélèvement.
La partie droite est remplie au moment du comptage.

TABLEAU I

Protocole pour une série de 200 dosages

	Tubes contrôle	Autres tubes	
Courbe standard		Traceur	1, 3, 5
Tubes 1 à 25	2, 4, 10, 15, 20	Excès d'anticorps anti-insuline	25
		Standard	Autres tubes
Plasma Tubes 26 à 200	30, 40, 50..., 190, 200	Plasmas à doser	Autres tubes (157 tubes en tout)

Les nombres indiquent les ordres de passage des tubes dans le compteur au cours du dosage. Les traceurs sont les tubes contenant l'hormone radioactive et l'immunsérum à la dilution utilisée pour le dosage. Les « standard » contiennent en plus l'hormone en quantité variable.

TABLEAU II

Résultats pour l'établissement de la courbe standard concernant l'exemple traité

Dose d'insuline µU/ml de plasma	0	3,125	6,25	12,5	25	50	100	200	400
Pourcentage d'activité adsorbée sur le silicate (P/T)	40,1 39,7 41,1	42,8 41,2	43,5 43,2	47,5 48,4	56,3 57,3	69,5 71,3	82,6 82,8	92,7 93,7	98,4 99,8

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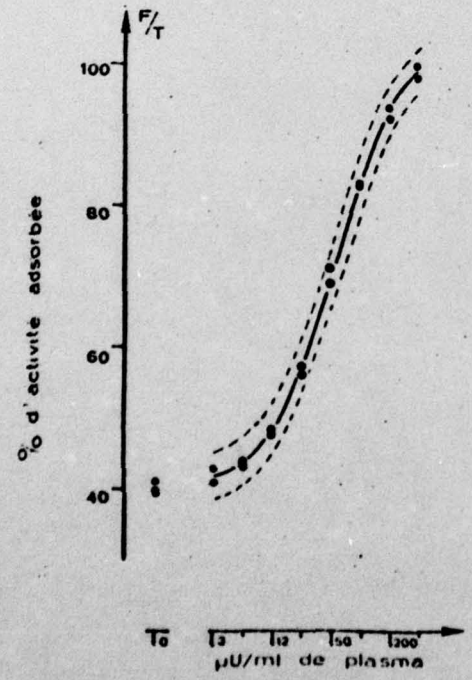

Fig. 3. — Courbe standard. Les points sont les points expérimentaux. La courbe en trait plein est la courbe calculée. Les courbes en pointillés sont les limites de précision (95 p. cent) de la courbe standard.

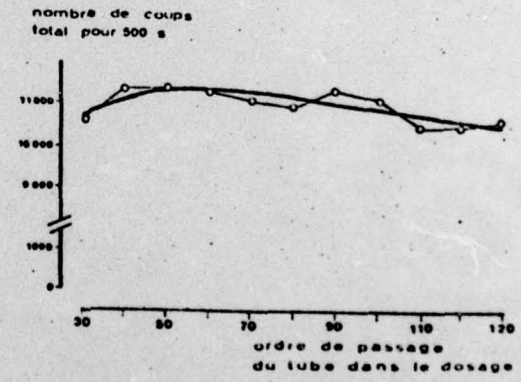

Fig. 4. — Variation de la mesure de la radioactivité des tubes de contrôle. La courbe en trait plein est la courbe calculée servant à calculer la valeur de référence (T) pour chaque tube à doser.

```
                    DATE DU PRELEVEMENT   7 AVR 70

COURONNE NO 1     NOM  PETIT        MAX                  NO ENQUETE 70-625 ( R 24 )
 TROU  NO  39     TEMPS   HPO 2M

-      QUANTITE D INSULINE ESTIMEE : 63.5  MIU/ML    ( 56. - 73. )

                                                    RAPPEL
                                          NBRE DE COUPS          8328.0
                                          CONTROLE ESTIME       11151.5
                                          CONTROLE PRECEDENT    10664.
                                          CONTROLE SUIVANT      11405.
ATTENTION : LES CONTROLES ENTOURANT CE TUBE DIFFERENT DE PLUS DE 5 P. CENT  REVOIR LE RESULTAT
```

```
                    DATE DU PRELEVEMENT   7 AVR 70

COURONNE NO 1     NOM  PETIT        MAX                  NO ENQUETE 70-625 ( R 26 )
 TROU  NO  41     TEMPS   HPO 3M

-      QUANTITE D INSULINE ESTIMEE : 37.3  MIU/ML    ( 33. - 42. )

                                                    RAPPEL
                                          NBRE DE COUPS          7446.0
                                          CONTROLE ESTIME       11207.4
                                          CONTROLE PRECEDENT    11 05.
                                          CONTROLE SUIVANT      11464.
```

```
                    DATE DU PRELEVEMENT   6 AVR 70

COURONNE NO 1     NOM  MARTINAU     PARCEL               NO ENQUETE 70-626 ( R 29 )
 TROU  NO  44     TEMPS   HPO 0M

TUBE VIDE  QUANTITE D INSULINE INFERIEURE A 3.0  MIU/ML  (0000 -0000 )

                                                    RAPPEL
                                          NBRE DE COUPS          2052.0
                                          CONTROLE ESTIME       11275.7
                                          CONTROLE PRECEDENT    11405.
                                          CONTROLE SUIVANT      11364.
```

Fig. 5. — Exemple de résultats « détaillés ». Le tube 39 était dans une zone où les contrôles variaient de façon jugée trop importante. Le tube 44 avait été signalé au moment du prélèvement comme « tube vide ».

RESULTATS RESUMES

DATE DU DOSAGE : 11 JUIN 70-1

HORMONE TODEE
DATE 4 JUIN 70 ACTIVITE SPECIFIQUE 200MIC/MIG

INSULINE
DATE 2-09-1966 SPECIFIC:. AC 41

ALBUMINE
DATE - - - NO DU LOT B 356

HORMONE LIBRE
DATE FEVRIER 70 NO DU LOT#18866

DATE PRELEVT	NOM MALADE	PRENOM	NO ENQUETE	NO USS	TEMPS PRELEVT	INCIDENT		QTE D INSULINE	COURONNE	TROU
6 AVR 70	AMASSIER	J-L	70-624	A 11	HPO 0M			19.5	1	P6
6 AVR 70	AMASSIER	J-L	70-624	A 12	HPO 30			57.5	1	P7
6 AVR 70	AMASSIER	J-L	70-624	A 13	HPO 1H			108.3	1	P8
6 AVR 70	AMASSIER	J-L	70-624	A 14	HPO 90			117.0	1	P9
6 AVR 70	AMASSIER	J-L	70-624	A 16	HPO 2H		A VERIFIER.	94.5	1	31
6 AVR 70	AMASSIER	J-L	70-624	A 17	HPO 3H		A VERIFIER.	58.1	1	32
6 AVR 70	AMASSIER	J-L	70-624	A 18	HPO 4H		A VERIFIER.	47.5	1	33
6 AVR 70	AMASSIER	J-L	70-624	A 19	HPO 5H		A VERIFIER.	43.3	1	34
7 AVR 70	PETIT	PAL	70-625	B 20	HPO 0M	-	A VERIFIER.	0.0	1	35
7 AVR 70	PETIT	PAL	70-625	B 21	HPO 30	-	A VERIFIER.	39.1	1	36
7 AVR 70	PETIT	PAL	70-625	B 22	HPO 60	-	A VERIFIER.	77.3	1	37
7 AVR 70	PETIT	PAL	70-625	B 23	HPO 90	-	A VERIFIER.	94.0	1	38
7 AVR 70	PETIT	PAL	70-625	B 24	HPO 2H	-	A VERIFIER.	69.5	1	39
7 AVR 70	PETIT	PAL	70-625	B 26	HPO 3H	-		37.3	1	41
7 AVR 70	PETIT	PAL	70-625	B 27	HPO 4H	-		21.0	1	42
7 AVR 70	PETIT	PAL	70-625	B 28	HPO 5H	-		10.0	1	43
6 AVR 70	MARTINAU	PARCEL	70-626	A 29	HPO 0M	TUBE VIDE	A VERIFIER.	0000	1	44
6 AVR 70	MARTINAU	PARCEL	70-626	A 30	HPO 30	-		5.7	1	45
6 AVR 70	MARTINAU	PARCEL	70-626	A 31	HPO 1H	-		21.0	1	46
6 AVR 70	MARTINAU	PARCEL	70-626	A 32	HPO 90	V		9.0	1	47
6 AVR 70	MARTINAU	PARCEL	70-626	A 33	HPO 2H	V		87.0	1	48

Fig. 6. — Exemple de résultats « résumés ». Au début, identification de la série de dosages ; les listes de résultats sont accompagnés de messages éventuels invitant à des vérifications.

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